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STRUCTURE ELUCIDATION OF STREPTINDOLE, A NOVEL GENOTOXIC METABOLITE ISOLATED FROM INTESTINAL BACTERIA

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Abstract: The structure of genotoxic metabolite, streptindole, isolated from intestinal bacteria (Streptococcus faecium IB 37) has been established as 2,2-di(3-indolyl) ethyl acetate by spectroscopic and synthetic methods.

In the recent past, much attention has been focused on the correlation between intestinal microflora and formation of colon cancer, which is supposed to be caused by carcinogens and/or cocarcinogens produced from dietary components by intestinal bacteria[1]. However, no clear evidence has yet been obtained. A number of laboratories are trying to characterize mutagenic and genotoxic metabolites of intestinal bacteria[2)-4)] because the cancer-causing substances would be detected by bacterial mutagenicity systems[5)6)]. Recently, we found that genotoxic metabolites are excreted from intestinal bacteria into the liquid media[7)]. We now report structure elucidation of a novel genotoxic metabolite produced by Streptococcus faecium IB 37 by means of the rec-assay with B.subtilis[8)].

The strain S.faecium IB 37, one of the predominant strains in human faeces[9)10)], was cultured in 30 liters of the modified EG medium[3)], filtered and the filtrate extracted with ethyl acetate. Fractionation of the ethyl acetate extract, guided by the rec-assay, was carried out on a silica gel column, followed by preparative HPLC [column; Develosil 60 (10/20), solvent system; n-hexane- ethyl acetate (2:1), Detector; $UV_{254\ nm}$ and RI]. Purified genotoxic metabolite (yield; 2.3 mg), streptindole (1), has the molecular formula of $C_{18}H_{20}N_2O_2$ (m/z 318.1376, determined by high-resolution mass spectrometry), λ_{max}^{EtOH} 273, 283 and 290 nm (ε=6.1x10^3, 6.5x10^3 and 5.8x10^3, respectively) and

$\gamma_{max}^{CHCl_3}$ 3400 (=NH), 1730 (acetyl) and 1620 cm^{-1} (double bond). These data suggested that streptindole has an indole ring in the structure and support for the assigned structure is obtained mainly from ^1H n.m.r. spectrum (d_6-acetone, δ; ppm).

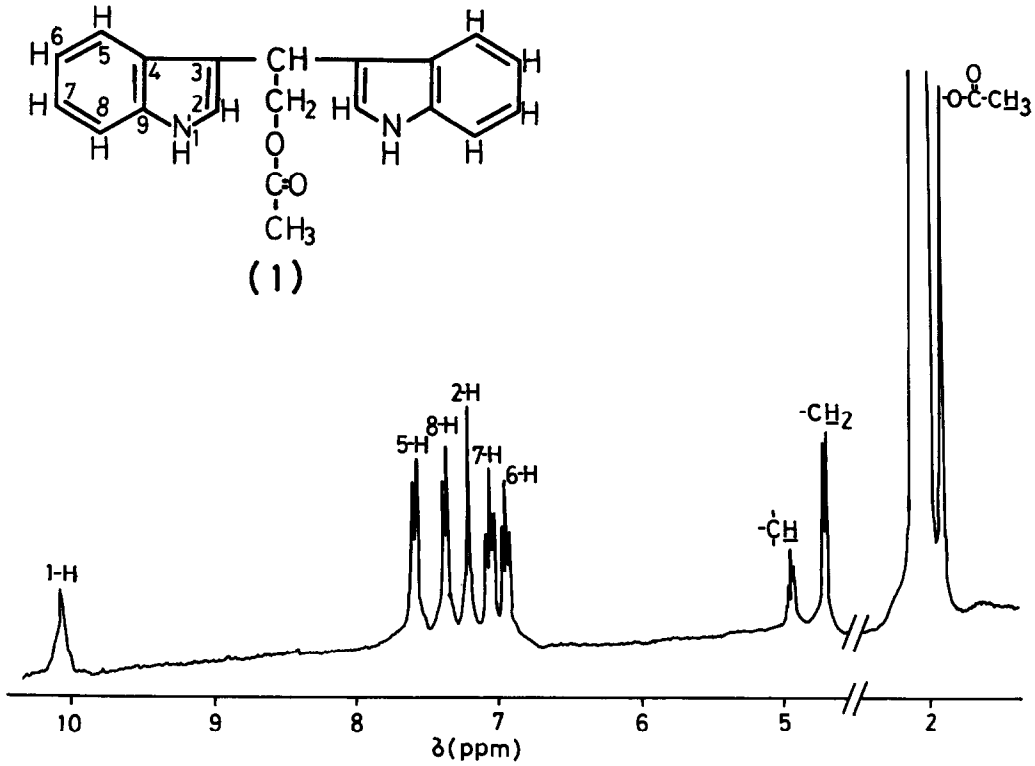
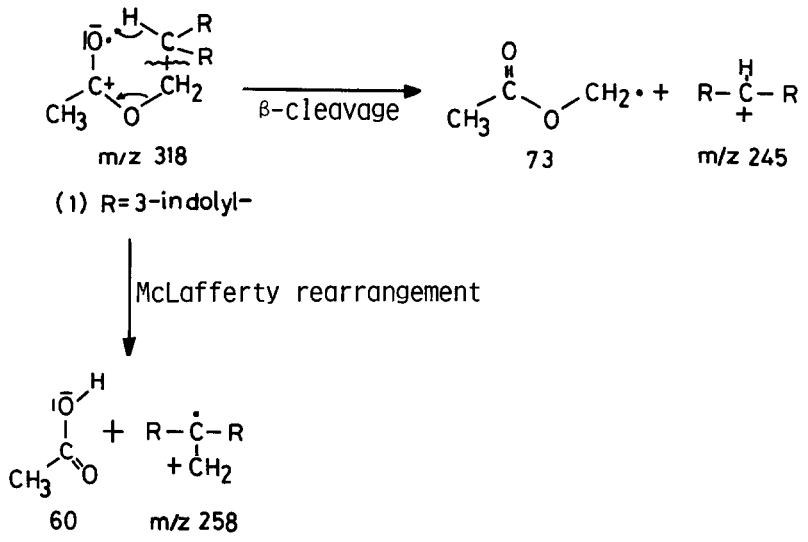


Fig.1 360 MHz ^1H n.m.r. spectrum of Streptindole (1) in d_6-acetone.

As shown in Fig.1, acetyl group was observed at δ 1.92 (3H, s) and methylene protons at δ 4.72 (2H, d, J=6.9 Hz) were found to be coupled with the methine proton at δ 4.93 (1H, t, J=6.9 Hz) and substituted ethyl acetate has been proposed as the partial structure of streptindole. Aromatic 10 protons located between δ 6.90 and 7.70 and two aromatic amine protons at δ 10.06 (2H, s) were supposed to be due to two symmetric 3-substituted indole rings. 2-H was observed at δ 7.23 (2H, s) and 6-H at δ 6.96 (2H, dd, J=6.8 and 7.8 Hz) was found to be coupled with 5-H at δ 7.62 (2H, d, J=7.8 Hz) and with 7-H at δ 7.08 (2H, dd, J=6.8 and 8.3 Hz). 7-H was also found to be coupled with 8-H at δ 7.40 (2H, d, J=8.3 Hz). Analysis of the mass spectrum of streptindole indicated that two 3-indolyl groups were substituted at 2-position of ethyl acetate, with significant fragment ions of m/z 258.1181 ($C_{18}H_{14}N_2$) and 245.1085 ($C_{17}H_{13}N_2$) (Scheme 1).



(Scheme 1)

From these data, structure of (1), 2,2-di(3-indolyl) ethyl acetate, has been proposed for streptindole. In order to confirm the structure of streptindole, synthesis of (1) has been carried out. 1 mmol of glycolaldehyde (60 mg) was dissolved in 30 ml of acetic anhydride and kept at room temperature overnight because glycolaldehyde exists in the solid state as the dimer[11]. After conversion to monomer in acid condition, glycolaldehyde was acetylated with 1 mmol of p-toluenesulfonic acid (190 mg). The acetylated glycolaldehyde was reacted with 2 mmol of indole (235 mg) without purification at room temperature for 5 hours, followed by purification using preparative TLC and HPLC. The purified synthetic compound showed chromatographic behavior and spectroscopic data identical to that of authentic streptindole, though the yield is only 2 % at the present stage. We are making our efforts to improve the yield of streptindole to determine its specificities of biological activity using various assay systems.

Streptindole is the first genotoxic di-indole type metabolite produced by intestinal bacteria. It showed a dose response in the range of concentration from 30 μg/plate to 600 μg/plate by the rec-assay and exhibited 5.3 mm of the differential growth inhibition between the H17 Rec^+ (wild) and M45 Rec^- (recombinationless) at a concentration of 300 μg/plate. At the present stage, we only observe DNA-damaging activity and genotoxicity which are reparable in B. subtilis cells. It is probable that streptindole is causing non-mutagenic lethal damage, however, usually more than 80% of the rec-assay positive chemicals are

mutagens, giving positive results in Salmonella and other test systems[6)13)]. Therefore, we are now continuing our efforts to determine mutagenic specificities of streptindole. We also obtained some evidences that other indole type genotoxic substances might be present in the metabolites produced by Veillonella parvula ATCC 10790 which is also predominant in human faeces, however, streptindole was not detected[12)]. Correlation between the excretion of the genotoxic metabolites and causation of colon cancer is still unknown. Further investigations of the isolation and identification of these genotoxic metabolites to show any correlation with colon cancer formation is being undertaken.

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